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Preface

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Acknowledgment

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Contents

Tables

Figures

Abstract

Microbial response was studied in four cold desert surface soils following moist soil incubation. Soils were typical Antarctic dry valley saline sands, low in organic matter content and low in abundances and kinds of viable microorganisms. Moist soil incubation increased the viable counts of three of the four soils. Most of the bacteria could grow at temperatures of 8°C; however, they grew more rapidly at 25°C. Failure of isolants from three of the soils to grow in sea salts medium indicated that they were probably not marine contaminants. It is suggested that the organisms in the three soils are probably indigenous organisms. They have adapted to the cold desert Antarctic terrestrial ecosystem, which provides a soil microbial ecology as a Mars model.

Growth of Bacteria in Soils from Antarctic Dry Valleys

I. Introduction

Before investigations are undertaken for detection of possible microbial life in a Martian soil ecosystem, studies of soils are being conducted in naturally harsh terrestrial environments, as well as simulated harsh environments (Ref. 1). The Antarctic cold desert is undoubtedly a region of major importance for these studies. It is an isolated region only recently invaded by man. It also has a simple microbial ecosystem uncomplicated by higher plants and animals, and it approaches the Martian environment in some of its characteristics, thereby providing a Mars model.

Many of the soil samples collected from the cold, barren, wind-swept, arid Antarctic dry valleys appear to have very low or undetectable populations of microorganisms, as determined by cultural methods (Refs. 2 through 8). By means of pour- and spread-plate techniques, viable counts were oftentimes less than 10 to 100 organisms/g of soil. Metabolic techniques ($^{14}CO_2$) also substantiated the low number of microorganisms in these soils (Refs. 9 and 10). However, because of contamination possibilities, it is never absolutely certain whether the colonies obtained from collected samples originated from growth of indigenous microorganisms. In addition to the possibility of laboratory contaminants, the organisms growing from these soils could have been derived from field party personnel or equipment utilized during sampling procedures, or from some other source in the natural environment, such as skuas. Nevertheless, checks were made for possible bacterial contamination by the procedure of incubating soils on desoxycholate agar plates at 20 and 37°C, and trypticase soy agar at 37, 45, and 55°C. Most Antarctic microorganisms grow best at 25°C or below,[1] and are not likely to survive following incubation at temperatures above 30°C.

Sources of microbial contamination from the natural environment have been considered previously (Refs. 2 and 11). A study of aerial microbial contamination was made in the Antarctic Peninsula (Refs. 12, 13, and 14). Aerial contamination has been found in falling snow at Byrd Land (Ref. 15). Exposure plates for bacteria and fungi exposed for several hours during a snow storm in Wheeler Valley did not yield any growth of colonies.[2]

A previous report for aerial contamination for a 2-h fallout period in McKelvey Valley suggested that there was a continual influx of airborne contaminants blowing into the valley from the sea, but local contamination, because of disruption of fines beneath the desert pavement, served as the primary source of aerial microflora (Ref. 16). Results obtained at Snow Hill Island (Ref. 12), showed a fallout rate of 1 bacterium/2 h exposure of petri plates. Repeated tests with an Anderson air sampler did not yield any microorganisms at a landlocked saline lake (Don Juan Pond) in the South Fork of Wright Valley (Ref. 17). Studies by Prof. R. E. Benoit in Taylor Valley have shown that the abundance of bacteria increases with onset and duration of the Antarctic austral summer; the peak abundance of bacteria, although low, were obtained

[1]Cameron, R. E., personal communication with Johnson, R. H., Arizona State University, Tempe, Ariz.

[2]Cameron, R. E., King, J., and David, C. N., unpublished data compiled during Wheeler Valley field trip, December 1967 through January 1968.

during midsummer, and the kinds of bacteria were essentially the same as the populations occurring in the soil where the air sampling was undertaken.[3] Bacterial air sampling made in the Transantarctic Mountains, approximately 325 km north of the South Pole, yielded few or no bacteria (Ref. 18). The bacteria were either aerial contaminants from a local field camp, or they were the same populations occurring in the surrounding soil landscape.

The phenomenon of increase in microbial activity or numbers is of special interest from the ecological aspect of terrestrial microbial ecology as well as from an extraterrestrial viewpoint. The microorganisms which grow following the wetting of dry soils would presumably be those which could also grow *in situ* if the soils were wetted by meteoric precipitation, flooding, irrigation water, glacial melt, or other source of moisture. If growth is obtained, then these microorganisms also must be capable of obtaining their nutritional requirements (except possibly fixed nitrogen) from the soil particles or solutions which are present at a given time, when the Antarctic temperature-moisture regime (solclime) is favorable, and they would be survivors of the indigenous or similar environment.

Growth, reproduction, or metabolic activity of microorganisms has oftentimes been observed following rewetting of air-dried, semi-arid, arid, or desert soils (Ref. 19). In some laboratory experiments, these observations were the result of determining the effect of water on microbial populations (Refs. 20 through 25). For other experiments, observations were made on controls while determining the effect of additives on the microbial population (Refs. 26 through 36). Long-term moist soil incubation of surface Taylor Valley soils incubated at variable room temperatures also have shown increases in viable counts with time, compared to initial counts obtained from the soil at a relatively dry *in situ* moisture content (Ref. 21).

Methods used for short-term moist incubation of dry, hot, and cold desert soils enriched with ^{14}C-labelled substrates, also have shown an increase in microbiological activity through detection of $^{14}CO_2$ evolution (Refs. 9, 10, 37, and 38), and are being considered for incorporation into extraterrestrial life-detection experiments.

It has been suggested that sea water media be used to determine whether Antarctic microorganisms are of marine origin (Ref. 11). Sea water enrichment cultures have been used for the isolation of soil phycomycetes from the South American Quadrant of Antarctica (Ref. 39). Bacterial isolants from the vicinity of penguin rookeries have been found to grow as well, if not better, at the salinity of sea water (Ref. 11). Saline soils from Taylor and Beacon Valleys, and soils and water from Don Juan Pond, Wright Valley, have been tested with various salt organic and soil extract enrichment media (Ref. 40); no microorganisms could be isolated from samples of Don Juan Pond *per se*, and although some of the saline soils were abiotic, others yielded halotolerant colonies on organic media with 5% added NaCl. It was concluded that the nearness of the sea to the Taylor Valley site, and the isolation of the bacteria from NaCl enrichment media, were sufficient for demonstrating the marine origin of the bacteria.

The purpose of this experiment is to determine whether evidence could be obtained to support the suggestions that microorganisms in Antarctic dry valley soils are indigenous to the soils from which they are isolated. The probability that these organisms are indigenous should be increased if it can be demonstrated that growth occurs following wetting of the soil, and that the microorganisms would not grow in the nearest less-harsh environment, i.e., sea water or other saline environments such as in some of the dry valley lakes and ponds. This report describes experiments attempting to clarify the above relationships of microorganisms in four Antarctic dry valley soils of known location and properties.

II. Materials and Methods

A. Soil Properties

Soil samples were collected from four different Antarctic dry valleys, west of McMurdo Sound and not in valleys open to the sea (Fig. 1). They were procured from McKelvey Valley (Fig. 2), and the Beacon Valley complex, including Arena (Fig. 3), Beacon (Fig. 4), and "No Name" (Fig. 5) valleys.[4] At each site, surface samples were collected aseptically by methods previously developed for desert soils (Ref. 41) and the samples maintained below freezing until used (Ref. 42).[5] These soil samples were selected for this experiment because of their location in some of the least-favorable habitats in the Antarctic dry valley region (Ref. 6), and because of their low initial viable microbial count. The microbial ecosystems were too harsh for algae, fungi, lichens, or mosses (Ref. 4).

[3]Cameron, R. E., personal communication with R. E. Benoit.

[4]"No Name" Valley: Unofficial Name.

[5]Samples collected for JPL desert microflora program by G. B. Blank, R. E. Cameron, and H. P. Conrow, Antarctic austral summers 1966–1967.

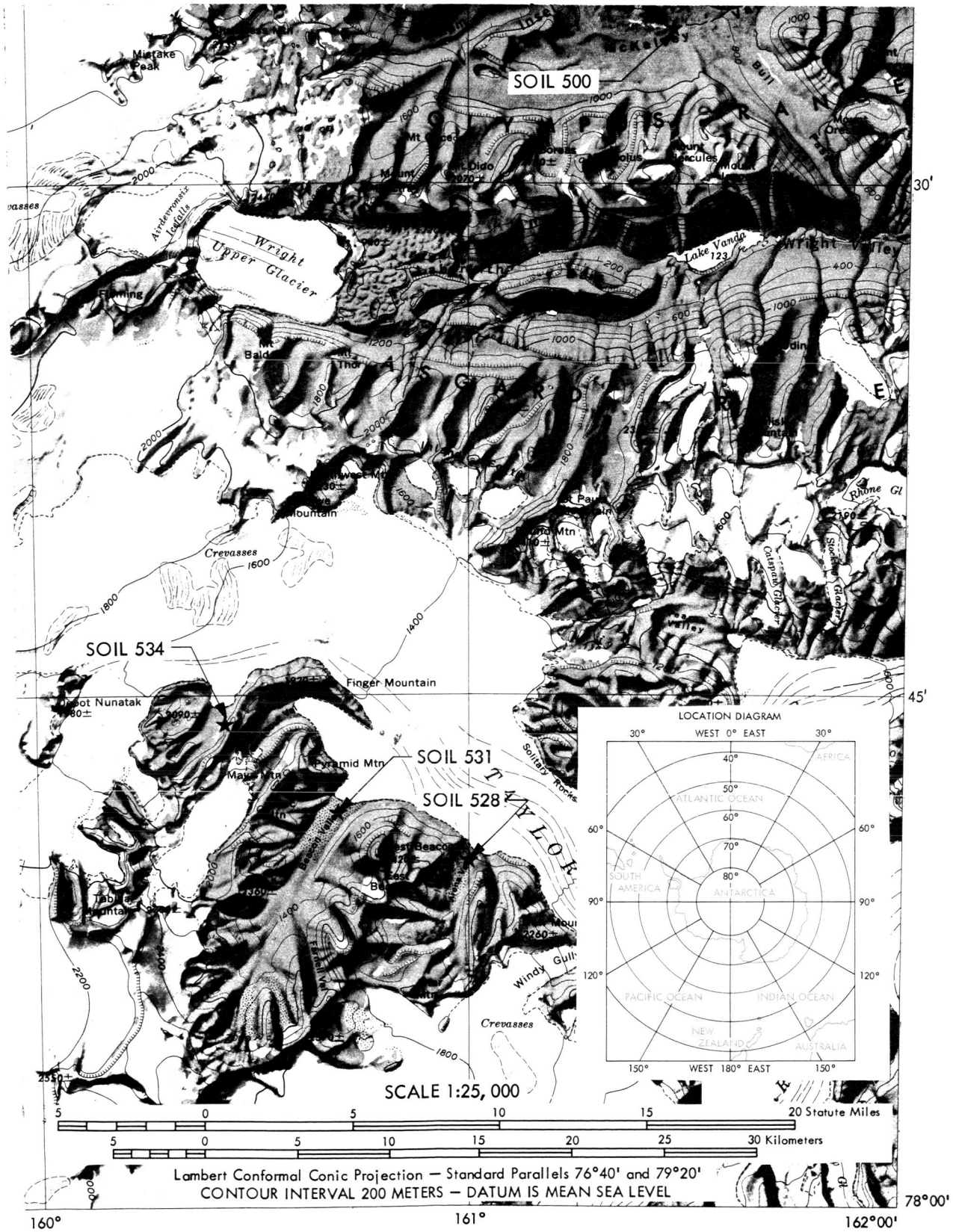

Fig. 1. Location of sample sites, Antarctic dry valleys, southern Victoria Land

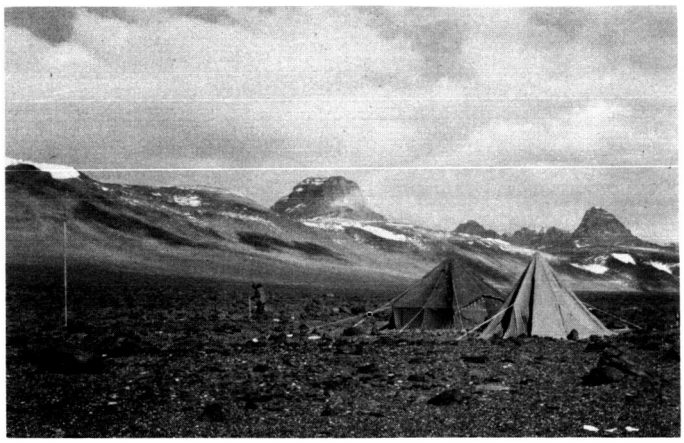

Fig. 2. McKelvey Valley sample site 500, elevation 800 m

Fig. 3. Arena Valley sample site 528, elevation 1400 m

Fig. 4. Beacon Valley sample site 531, elevation 1400 m

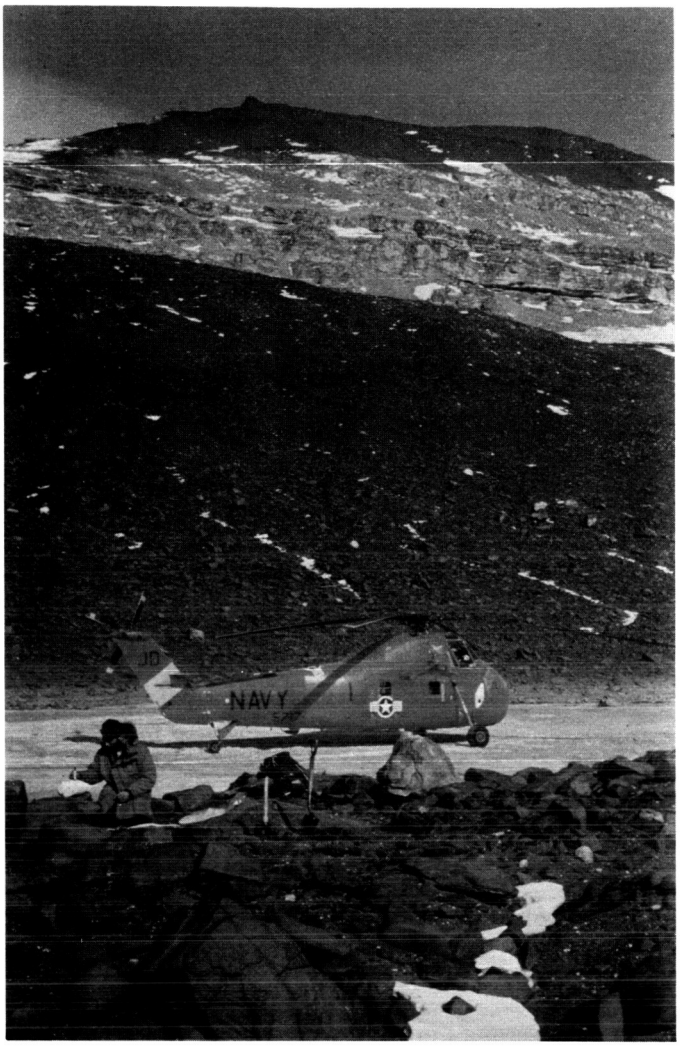

Fig. 5. "No Name" Valley sample site 534, elevation 1500 m

Some of the pertinent soil physical, physico-chemical, and chemical properties are shown in Tables 1 and 2. These analyses were obtained primarily on the sieved ($\leq$2 mm) air dry soils by methods indicated previously (Ref. 4). As shown by these analyses, all of these samples are typical, poorly developed cold-desert or frigic soils (Refs. 43 and 44). The soils are mostly coarse-textured, brownish lithochromic dry sands with pH values, electrical conductivities, and predominances of Na^{+}, Ca^{++}, Mg^{++}, Cl^{-}, $SO_4^{=}$, NO_3^{-} on the exchange complex, indicative of saline desert soils. Cation exchange values compare favorably with those obtained for other cold-desert soils as well as southwest U.S. desert soils (Ref. 4). Nitrate is the major anion in three of these soils, in contrast to soils obtained from Wheeler Valley, which has a much more

Table 1. Physical and physico-chemical properties of Antarctic dry valley soils

Soil	Sample depth, cm	Location	Texture	Color and Munsell notation air dry	*In situ* moisture content, wt. %	pH, saturated soil paste	Electrical conductivity, $\times 10^{-6}$ mhos/cm^2 at 25°C	Cation exchange capacity, meq/100 g
500	Surface 5	McKelvey, near center of valley	Loamy sand	10 YR 6/4 light yellowish brown	1.4	8.0	3360	3
528	Surface 3	Arena, ~0.5 km south of Taylor Glacier	Loamy sand	10 YR 5/3 brown	0.48	6.7	660	7
531	Surface 3	Beacon, ~0.5 km south of Taylor Glacier	Sand	10 YR 6/3 pale brown	1.7	7.3	880	7
534	Surface 3	"No Name,"[a] west of Turnabout, near small frozen pond	Loamy sand	10 YR 6/4 light yellowish brown	1.6	7.2	875	6

[a]Unofficial name.

Table 2. Chemical properties of Antarctic dry valley soils

Soil	Organic C, wt. %	Organic N, wt. %	Carbonate C, wt. %	Ions, ppm in 1:5, soil: H_2O extract									
				Na^+	K^+	Ca^{++}	Mg^{++}	NH_4^+	Cl^-	$SO_4^=$	HCO_3^-	NO_3^-	$PO_4^{\equiv}$
500	0.09	0.007	0.01	650	5	190	71	0.0	665	510	24	780	0.1
528	0.01	0.003	<0.01	97	6	36	15	0.1	66	135	12	200	0.1
531	0.01	0.002	<0.01	68	3	8	5	0.2	37	20	24	100	0.0
534	0.08	0.012	0.01	57	5	120	31	0.1	100	350	12	160	0.1

favorable environment (Ref. 46). Organic C and N are moderately high for two of the soils (500 and 534), as compared to other dry valley soils, but soil samples 528 and 531 have low values.

B. Microbiological Methods

Initial viable counts were performed at JPL. These were determined by sprinkle or spread plate and dilution tube methods described previously (Refs. 1, 4, and 46). In addition to trypticase soy agar (TSA, Baltimore Biological Laboratory), organic-salt medium (STV), with salt composition based on soil, extract composition also was used (Refs. 5 and 40). Counts were made after from 3 to 6 weeks incubation in high humidity at 18°C.

The following procedure was used for the moist soil incubation experiments. Ten grams of a sample were placed aseptically into a sterile 25-mm screw cap test tube and 5 ml of sterile distilled water were added. The sample was suspended by thoroughly stirring on a Vortex mixer, and 0.5 ml of the suspension was removed for decimal serial dilutions in sterile distilled water. Aliquots of the dilutions (1/10 ml) were streaked on 1% trypticase soy broth (TSB) agar plates and incubated at either 8 or 25°C for 4 weeks. Four plates were made from each dilution tube with distilled water added.

Dilution tubes or petri plates, which were used to obtain growth or viable counts at 8°C, were preincubated at this temperature before being inoculated. After the initial viable count was thus made, the samples in the screw cap tubes were incubated in the dark at 8 or 25°C, and sampled at 5, 7, 20, and 40 days after incubation. Three tubes of sample 500 and four tubes of soil samples 528, 531, and 534 were incubated at 8 and at 25°C.

Growth of isolated microorganisms was determined at 5 and 20°C in TSB liquid medium in tubes with and without the addition of sea salts (Ref. 47).

III. Results and Discussion

A. Initial Viable Counts

The initial viable counts and those recorded at the beginning of the incubation experiment are listed in Table 3. Although there are some differences between the initial viable counts performed at JPL and those obtained at the beginning of the incubation experiment, these differences are relatively insignificant when compared to viable counts from other desert soils which are orders of magnitude higher. Since the conclusion of this experiment, it has also been determined that sample viability must be maintained near −30°C (Ref. 1). The samples used for the tests in this experiment were obtained from a −5°C storage chamber. A much longer incubation period could possibly increase the viable counts (Refs. 3 and 48). The results also suggest that low viable counts are not because of injury or death of psychrophilic organisms due to exposure to higher temperatures, i.e., 25°C. However, some Antarctic soils are toxic because of soluble salts and especially boron, as shown by viable counts obtained after abiotic and viable soils had been mixed together in a 5:1 water solution for less than 6 h (Ref. 5).

Table 3. Initial viable counts in colonies per gram of soil at JPL and at beginning of incubation period

Soil	JPL				Experimental	
	2°C	20°C	2°C	20°C	8°C	25°C
500	15	25	5	0	<10	<10
528	60	140	65	0	<10	<10
531	670	580	0	1900	<10	310 ±46
534	1280	2900	430	1330	<10	<10
Media	TSA		STV		1% TSB agar	

B. Changes in Viable Counts

Changes in the viable counts of soils 500, 531, and 534 in incubation with water are given in Fig. 6. The viable counts obtained from these wetted soils increased with time. The results with soils 500 and 534 suggest that the microorganisms were ordinary facultative organisms which can grow at 8°C, but grow faster at 25°C. In soil 531, some of the microorganisms that grew in the soil at 25°C could not be isolated at 8°C and may have been obligate mesophiles. For these three soils, the viable counts from the replicate tubes were within an order of magnitude; this was considered adequate for replications, and only the average counts are given. With soil 528, however, the replicate tubes varied considerably (Table 4). One tube (No. 2), incubated at 25°C, gave results similar to the other three soils while viable counts greater than 10/g were never obtained from tube No. 3. Some organisms apparently grew in the soil at 8°C, but were subsequently isolated only when the agar plates were incubated at 25°C.

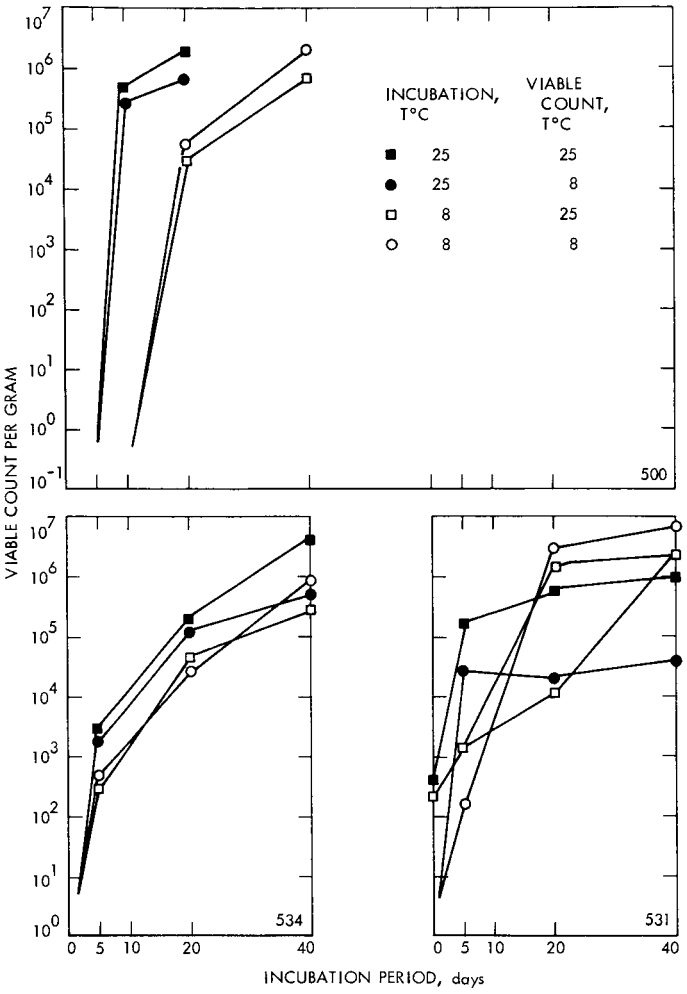

Fig. 6. Growth of selected bacterial isolants in sea salts medium

Table 4. Viable counts from soil sample 528, per gram × 10^3

Tube incubation temperature, °C	Tube No.	Plate incubation temperature, °C							
		25°C				8°C			
		0	5	20	40	0	5	20	40
25	1				1200				
	2		58	187	266		49	180	208
	3								
	4				2				
8	5				9				
	6			4	40				44
	7				7				
	8				6				

C. Isolants Obtained From Soil-Water Incubation

Pure cultures were obtained from the agar plates made from the 40-day sampling of soils 528, 531, and 534. Since it was impossible to maintain all of the colonies on these plates, colonies which appeared distinctive were selected, and it was assumed that all of the different organisms in these soils in high numbers were represented. Seventeen bacteria were isolated; none formed spores and all were *Micrococcus* sp., coccoid or pleomorphic rods, typical of various soil diphtheroids, e.g., *Mycococcus* sp., *Brevibacterium* sp., *Mycobacterium* sp., *Corynebacterium* sp., and *Arthrobacter* sp. (Ref. 1). Twelve of the isolated colonies were yellow, pink, or orange on 1% TSB-agar plates. Table 5 indicates which of the isolates were able to grow in a sea salts medium.

IV. Concluding Remarks

The variability of the replications, and the ability of all of the organisms in soil 528 to grow in sea water, suggest that these organisms are more likely marine contaminants than part of an indigenous flora. This soil is no more salty than soils 531 and 534, and considerably less salty than McKelvey soil 500. The microorganisms which grew in soils 500, 531, and 534 are more likely to be indigenous organisms because reproducible growth was obtained in all moist soil incubation replications. They were probably not of recent marine origin and should not be considered as contaminants. They have adapted to the cold desert soils. Additional tests in a sea salts medium, other salt media and moist soil incubations may indicate whether or not other Antarctic soils also may contain indigenous populations or recent contaminants.

Table 5. Growth of isolates in TSB with sea salts

Soil	Isolate	Temperature of sea salts medium	
		5°C	20°C
528	A	+	+
	B	+	+
	C	+	+
531	A	−	−
	B	−	−
	C	−	−
	D	−	−
	E	−	−
	F	+	+
	G	−	−
534	A	−	−
	B	−	−
	C	−	−
	D	−	−
	E	+	+
	F	−	−
	G	−	−

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16. Abstract

Microbial response was studied in four cold desert surface soils following moist soil incubation. Soils were typical Antarctic dry valley saline sands, low in organic matter content and low in abundances and kinds of viable microorganisms. Moist soil incubation increased the viable counts of three of the four soils. Most of the bacteria could grow at temperatures of 8°C; however, they grew more rapidly at 25°C. Failure of isolants from three of the soils to grow in sea salts medium indicated that they were probably not marine contaminants. It is suggested that the organisms in the three soils are probably indigenous organisms. They have adapted to the cold desert Antarctic terrestrial ecosystem, which provides a soil microbial ecology as a Mars model.

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